European Journal of Cancer Prevention 1995, 4, 469–474

Nitrosamine intake and gastric cancer risk

C La Vecchia[1,2], B D'Avanzo[1], L Airoldi[1], C Braga[1], A Decarli[2]

(Received 20 July 1995; accepted 20 September 1995)

The association between intake of *N*-nitrosodimethylamine (NDMA), the most commonly occurring of the volatile nitrosamines derived from foods, and gastric cancer risk has been investigated using data from a case–control study conducted in Northern Italy between 1985 and 1993, including 746 incident cases of gastric cancer and 2,053 controls admitted to hospital for acute, non-neoplastic and non-digestive tract diseases, not related to long-term modifications of diet. Information was collected on frequency of consumption of 29 food items, including selected sources of NDMA. Compared with subjects in the lowest tertile of NDMA intake, the odds ratios (ORs) were 1.1 in the intermediate and 1.6 in the highest tertile of intake. These estimates were not appreciably modified after allowance for total energy intake, other major dietary and non-dietary correlates of gastric cancer, and estimated intake of nitrite and nitrate: the multivariate OR for the highest NDMA intake tertile was 1.4 (95% CI 1.1–1.7). The association was consistent across strata of sex and age, but somewhat stronger in males and in subjects below age 60 (OR in the highest tertile, 1.8). Limitations of exposure assessment and absence of information on other *N*-nitrosamines preclude, however, any definite assessment of the possible role of exogenous *N*-nitrosamines in gastric carcinogenesis.

Key words: Case control study, diet, gastric cancer, nitrosamines.

Introduction

N-nitroso compounds, and in particular *N*-nitrosamines, may contribute to gastric carcinogenesis (World Health Organization, 1978). The possible effect of the main precursors of exogenous *N*-nitroso compounds, nitrites and nitrates, on the risk of gastric cancer has been investigated in a few epidemiological studies. Of these, some reported a direct association between consumption of nitrite-containing foods and gastric cancer (Risch *et al*, 1985; Buiatti *et al*, 1990; La Vecchia *et al* 1994), while another case control study (Boeing, 1991) and two ecological studies (Forman *et al*, 1985; Palli *et al*, 1991) found little or no evidence of association. Inconsistent associations of nitrate contained in food with gastric cancer risk have, however, been observed in a few studies (Forman *et al*, 1985; Risch *et al*, 1985; Buiatti *et al*, 1990; Boeing *et al*, 1991; Palli *et al*, 1991; Gonzàles *et al*, 1994). For instance, a German study found an odds ratio (OR) of 1.26 in the highest quintile of consumption of nitrate (Boeing *et al*, 1991), an Italian study an OR of 0.9 (Buiatti *et al*, 1990) and a study from Canada an OR of 0.66 in the highest quintile of intake, in the absence of significant trend in risk (Risch *et al*, 1985).

Only scattered data on the relationship between gastric cancer and biological indicators of endogenous *N*-nitrosamines have been published. A Polish study (Zatonski *et al*, 1989) found that endogenous *N*-nitrosamine formation was enhanced in individuals from high-risk areas for stomach cancer. In Costa Rica (Sierra *et al*, 1993) levels of intragastrically formed *N*-nitroso compounds were higher in subjects

[1]*Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea 62, 20157 Milan, Italy.* [2]*Istituto di Statistica Medica e Biometria, Università di Milano, Istituto Nazionale dei Tumori, Via Venezian 1, 20133 Milan, Italy. Correspondence to: B D'Avanzo. Fax: (+39) 2 354 6277*

from high-risk areas, while a study on nitrate and *N*-nitrosoproline excretion in two Italian regions did not find a consistent relationship with gastric cancer rates (Knight *et al*, 1992).

With reference to exogenous *N*-nitrosamines, no significant association was observed between *N*-nitrosodimethylamine (NDMA) and gastric cancer risk in a Canadian case control study (Risch *et al*, 1985) which gave an OR of 0.94, although with wide confidence intervals (0.1 6.1). A Spanish case control study (Gonzàles *et al*, 1994) provided a risk estimate, adjusted for calorie intake, of 2.09 in the highest quartile of *N*-nitrosamine intake. A study of 92 cases and 128 controls from Marseille, France (Pobel *et al*, 1995) found ORs of 4.1 and 7.0 for subsequent tertiles of NDMA intake. Due to the low number of cases, however, the confidence intervals were very wide (1.9 26.5 for the highest tertile).

In a paper on the effect of selected micronutrients on gastric cancer risk, we reported a slightly increased risk for nitrite intake, and a significant protection for nitrate (La Vecchia *et al*, 1994). Some estimates of direct individual exposure to *N*-nitrosamines from selected foods have recently become available (Gavinelli *et al*, 1988; Cornée *et al*, 1992). Surveys of *N*-nitroso compound content of foods have indicated NDMA as the most commonly occurring volatile nitrosamine (Ministry of Agriculture, Fisheries and Food, UK, 1987; Hotchkiss, 1989). Therefore, in this paper we deal with the effect of NDMA intake on gastric cancer risk.

Subjects and methods

Since 1985, we have been conducting a case–control study on gastric cancer in a network of hospitals in the Greater Milan area. As already described (La Vecchia *et al*, 1987, 1994), trained interviewers administered a structured questionnaire including questions on sociodemographic and anthropometric characteristics, smoking habits, frequency of intake of 29 selected foods (bread, pasta or rice, whole-grain bread or pasta, polenta, pastries, beef/veal, poultry, liver, raw ham, ham, salami and other sausages, canned meat, fish, milk, cheese, eggs, potatoes, pulses, cabbage and other cruciferae, spinach, lettuce, other green vegetables, tomatoes, carrots, peppers, apples, citrus fruit, melon, and other fresh fruit), alcohol consumption, intake of coffee and other methylxanthine-containing beverages, medical history (problem-oriented), and family history of selected cancers, obstetric and reproductive factors. The present analysis is based on data collected up until June 1993.

Cases were 746 patients (457 men and 289 women) with incident, histologically confirmed stomach cancer diagnosed no longer than 1 year before the interview, aged 19–74 years (median age 61), admitted to the National Cancer Institute and the Ospedale Maggiore of Milan, which includes the four largest teaching and general hospitals in the Greater Milan area.

Controls were 2,053 subjects (1,205 males and 848 females) aged 19 74 years (median age 55) admitted to the same network of hospitals for acute, non-neoplastic, non-digestive tract diseases, unrelated to long-term modifications of diet. Their major admission diagnostic categories were traumatic diseases (47%), non-traumatic orthopaedic conditions (such as low back pain and disc disorders, 20%), acute surgical conditions (19%), and other miscellaneous disorders, including skin, eye, ear, nose and throat conditions (14%). The catchment areas of cases and controls were comparable. Over 80% of cases and controls came from Lombardy region, and over 90% from Northern Italy. Less than 5% of patients approached for interview refused to participate.

The estimates of NDMA intake were derived from the content values measured by an Italian survey on selected foods (Gavinelli *et al*, 1988), or from other published data (Cornée *et al*, 1992).

Data analysis included computation of ORs, as estimators of relative risks, and the corresponding 95% confidence intervals (CI) (Breslow and Day, 1980) of gastric cancer for subsequent tertiles (defined on the controls) of NDMA intake. To allow for potential confounding factors, we fitted three models, one including only terms for age and sex; a second that also included terms for education, family history of gastric cancer, a combined food score (*ie* a variable combining high intake of cereals and starches with low consumption of fruit and vegetables; La Vecchia *et al*, 1987), and intake of ß-carotene, vitamin C and total calories; and a third that additionally included terms for nitrite and nitrate intake. For multiple levels of exposure, the significance of the linear trends in risk was assessed comparing the difference between the deviances of the models without and with the term of interest to the χ^2 distribution with one degree of freedom (Breslow and Day, 1980).

Results

The distribution of cases and controls according to sex, age and education is given in Table 1. Cases were somewhat older than controls and significantly less

educated, thus, allowance for these variables was made in all subsequent analyses.

Table 2 shows the distribution of cases and controls, and the corresponding ORs, in subsequent tertiles of NDMA intake. Compared with subjects in the lowest tertile of NDMA intake, the ORs were 1.1 in the intermediate and 1.6 in the highest tertile of intake. The ORs were significant in the highest tertile of intake, and adjustment for several potential confounding factors did not substantially modify any of the estimates. Likewise, after allowance for intake of nitrite and nitrate from foods, the estimates were not appreciably modified. The multivariate OR for the highest NDMA intake tertile was 1.4 (95% CI 1.1 1.7). The trends in risk were significant.

The effect of NDMA intake was further analysed in separate strata of sex and age (Table 3). The association was consistent across strata considered, but somewhat stronger in males and in subjects below age 60. For males and in subjects below 60 years of age the OR in the highest tertile was 1.8.

Table 1. Distribution of 746 stomach cancer cases and 2,053 controls in Milan, Italy, 1985 1993, according to socio-demographic characteristics

	Cases		Controls	
	No.	%	No.	%
Sex				
Male	457	61.3	1,205	58.7
Female	289	38.7	848	41.3
Age (years)				
< 50	129	17.3	695	33.9
50–59	209	28.0	604	29.4
60–69	296	39.7	597	29.1
≥ 70	112	15.0	157	7.6
Education (years)				
< 7	473	63.4	999	48.7
7–11	182	24.4	602	29.3
≥ 12	91	12.2	452	22.0

Discussion

This study found a moderate but significant association between exogenous NDMA intake and gastric cancer risk. The association was consistently observed across strata of sex and age.

The study was conducted in Lombardy, a high-risk area for gastric cancer, where incidence rates were around 30/100,000 males and 15/100,000 females (world standard) in the mid-1980s, *ie* one of the highest incidence rates in Western Europe (Levi *et al*, 1993).

These findings are thus of specific interest, since although the role of intragastrically formed *N*-nitrosamines on gastric cancer has been analysed in a few studies (Zatonski *et al*, 1989; Knight *et al*, 1992; Sierra *et al*, 1993; Gonzàles *et al*, 1994), the role of *N*-nitrosamines directly ingested from foods is still unclear.

The mean value of daily NDMA intake in this population was 0.18 μg. This is similar to those found in Canadian (Risch *et al*, 1985) and Spanish populations (Gonzàles *et al*, 1994), higher than in

Table 2. Distribution of 746 stomach cancer cases and 2,053 controls in Milan, Italy, 1985 1993, and odds ratios (ORs) with 95% confidence intervals (CIs) of gastric cancer according to tertiles of *N*-nitrosodimethylamine (NDMA) intake

	Cases		Controls		OR (95% CI)[a]		
	No.	%	No.	%	1	2	3
NDMA (μg/day)							
≤0.130	207	27.7	683	33.3	1[b]	1[b]	1[b]
0.131 0.190	231	31.0	687	33.5	1.13 (0.9 1.4)	1.12 (0.9 1.4)	1.11 (0.9 1.4)
≥0.191	308	41.3	683	33.3	1.56 (1.3 1.9)	1.41 (1.1 1.8)	1.37 (1.1 1.7)
χ^2 trend[a]					17.89[c]	9.11[c]	7.42[c]

[a] Estimates from multiple logistic regression equations including terms for: OR 1, age and sex; OR 2, age, sex, education, family history of gastric cancer, combined food score index, intake of β-carotene, vitamin C and total calories; 3, as OR2 plus nitrite and nitrate intake.
[b] Reference category.
[c] $P < 0.01$.

Table 3. Odds ratios with 95% confidence intervals and numbers of cases : numbers of controls for gastric cancer according to tertiles of daily intake of *N*-nitrosodimethylamine (NDMA) in strata of sex and age in Milan, Italy, 1985 1993

	Tertile of intake of NDMA			χ^2 trend[a]
	1st[b]	2nd	3rd	
Sex				
Male	1	1.17 (0.9 1.6)	1.75 (1.3 2.3)	17.83[c]
	[115 : 394]	[121 : 352]	[221 : 459]	
Female	1	1.06 (0.8 1.5)	1.28 (0.9 1.8)	1.86
	[92 : 289]	[110 : 335]	[87 : 224]	
Age (years)				
< 60	1	1.44 (1.0 2.0)	1.79 (1.3-2.4)	14.39[c]
	[82 : 431]	[104 : 399]	[152 : 469]	
> 60	1	0.90 (0.7 1.2)	1.37 (1.0-1.9)	4.24[d]
	[125 : 252]	[127 : 288]	[156 : 214]	

[a] Estimates from multiple logistic regression equations including terms for age and, when appropriate, sex.
[b] Reference category.
[c] $P < 0.01$.
[d] $P < 0.05$.

Swedish and Finnish ones (Boeing, 1991), but somewhat lower than estimates from Japan and the UK (Boeing, 1991), where the contributions of beer, cured meat and fish to NDMA intake were substantial.

These data are based on a single volatile *N*-nitrosamine identified in a few selected food items, in the absence of information on other non-volatile *N*-nitrosamines and other *N*-nitroso compounds produced intragastrically from nitrates and nitrites. However, even when we introduced an estimate of nitrite and nitrate intake into the model, the association with NDMA decreased only moderately, suggesting that any potential effect of exogenous NDMA on the risk of gastric cancer is, at least partly, independent from that of other sources of *N*-nitrosamines or *N*-nitrosamides in the stomach. Furthermore, NDMA can be considered a general indicator of exposure to other *N*-nitroso-compounds (Ministry of Agriculture, Fisheries and Food, 1987). However, we had no information on endogenous *N*-nitroso compound formation, which is influenced by gastric pH levels (Reed *et al*, 1981) and other complex factors, including microbial species in the mouth and stomach, *N*-nitrosation inhibitors, besides subjective individual variation (Xu and Reed, 1993).

Within the process of gastric carcinogenesis, it has been suggested that *N*-nitroso compounds and *N*-nitrosamines have a genotoxic effect (Mirvish, 1983; Correa, 1988), and hence a role in the early stages of the process (Day and Brown, 1980). The finding of a stronger effect in younger subjects is in agreement with this possible role of NDMA, because the effect on the relative risk of an initiating factor is more apparent in subjects at lower baseline risk (Day and Brown, 1980).

The apparently weaker association of NDMA intake with gastric cancer in women is in agreement with other reports, in which women showed the same risk pattern as men, but a somewhat weaker association with micronutrients, possibly on account of their generally more favourable dietary pattern (Buiatti *et al*, 1990; La Vecchia *et al*, 1994).

This was a hospital-based case–control study, and has the related strengths and weaknesses. With regard to the possible weaknesses of the study design, no distinction was made between various histological types of gastric cancer (intestinal and diffuse), nor between various anatomical locations, although these may recognize different aetiological mechanisms (Howson *et al*, 1986). Likewise, we had no information on *Helicobacter pylori* (Parsonnet *et al*, 1991; Muñoz, 1994) in cases and controls, although *Helicobacter pylori* antibody prevalence has not been shown to correspond to high-risk areas of gastric cancer in Italy (Palli *et al*, 1993).

Among the strengths of this study were the large dataset, the comparable catchment areas of cases and controls, the practically complete participation, as well as the absence of important confounding by a number of major known or likely correlates of gastric cancer risk. Control selection, moreover, was addressed to avoid inclusion of any chronic and

digestive-tract diseases, and of any condition related to long-term modification of diet. With reference to information bias, a systematic difference between cases and controls in reporting the food items' contribution to the estimation of NDMA intake is unlikely, since there was no general awareness of their possible effects on stomach cancer risk.

Some of the food items contributing to the estimated intake of NDMA are not frequently consumed, so there may have been some misclassification of the intake. If not differential between cases and controls, such a misclassification might have led to an underestimation of the true risk. The questionnaire was not validated, and the information on NDMA content was available only for a limited number of foods. Furthermore, we had no information on the nitrate content of water consumed in the region, and other *N*-nitrosamines, which are more difficult to measure and quantify (Preussmann, 1984), may have a role in gastric carcinogenesis (Druckrey *et al*, 1967). If levels of these *N*-nitrosamines are correlated to NDMA levels and not differentially reported by cases and controls, lack of information on them may lead to a systematic underestimation of true association.

In conclusion, these and other possible limitations of the study design, and uncertainties of exposure assessment do not totally eclipse the observation of a direct relationship between intake of NDMA and gastric cancer risk in this population.

References

Boeing H (1991). Epidemiological research in stomach cancer: progress over the last ten years. *J Cancer Res Clin Oncol* **117:** 133 43.

Boeing H, Frentzel-Beyme R, Berger M *et al* (1991). Case control study on stomach cancer in Germany. *Int J Cancer* **47:** 858 64.

Breslow NE, Day NE (1980). *Statistical Methods in Cancer Research.* Vol 1. The Analysis of Case Control Study (IARC Sci Publ No. 32). IARC, Lyon.

Buiatti E, Palli D, Decarli A *et al* (1990). A case control study of gastric cancer and diet in Italy. II. Association with nutrients. *Int J Cancer* **45:** 896 901.

Cornée J, Lairon D, Velema J, Guyader M, Berthezène P (1992). An estimate of nitrate, nitrite and nitrosodemethylamine concentrations in French food products or food groups. *Sci Aliments* **12:** 155 97.

Correa P (1988). A human model of gastric carcinogenesis. *Cancer Res* **48:** 3554 60.

Day NE, Brown CC (1980). Multistage models and primary prevention of cancer. *J Natl Cancer Inst* **64:** 977 89.

Druckrey H, Preussmann R, Ivankovic S *et al* (1967). Organotrope carcinogene Wirkungen bei 65 verschiedenen *N*-nitroso-verbindungen an BD-Ratten. *Z Krebsforsch* **69:** 103 201.

Forman D, Al-Dabbagh S, Doll R (1985). Nitrates, nitrites and gastric cancer in Great Britain. *Nature* **313:** 620 25.

Gavinelli M, Fanelli R, Bonfanti M, Davoli E, Airoldi L (1988). Volatile nitrosamines in foods and beverages: preliminary survey of the Italian market. *Bull Environ Contam Toxicol* **40:** 41 6.

Gonzáles CA, Riboli E, Badosa J *et al* (1994). Nutritional factors and gastric cancer in Spain. *Am J Epidemiol* **139:** 466 73.

Hotchkiss JH (1989). Preformed *N*-nitroso compounds in foods and beverages. *Cancer Surv* **8:** 295 321.

Howson CP, Hiyama T, Wynder EL (1986). The decline in gastric cancer: epidemiology of an unplanned triumph. *Epidemiol Rev* **8:** 1 27.

Knight T, Piratsu R, Palli D *et al* (1992). Nitrate and *N*-nitrosoproline excretion in two Italian regions with contrasting rates of gastric cancer: the role of nitrate and other factors in endogenous nitrosation. *Int J Cancer* **50:** 736 9.

La Vecchia C, Negri E, Decarli A, D'Avanzo B, Franceschi S (1987). A case control study of diet and gastric cancer in Northern Italy. *Int J Cancer* **40:** 484 9.

La Vecchia C, Ferraroni M, D'Avanzo B, Decarli A, Franceschi S (1994). Selected micronutrient intake and the risk of gastric cancer. *Cancer Epidemiol Biomarkers Prev* **3:** 393 8.

Levi F, La Vecchia C, Lucchini F, Boyle P (1993). Cancer incidence and mortality in Europe, 1983 87. *Soz Präventivmed* **3 (Suppl):** S155 229.

Ministry of Agriculture, Fisheries and Food (1987). *Nitrate, Nitrite and N-Nitroso Compounds in Food.* Her Majesty's Stationery Office, London.

Mirvish SS (1983). The etiology of gastric cancer. Intragastric nitrosamide formation and other theories. *J Natl Cancer Inst* **71:** 629 47.

Muñoz N (1994). Is *Helicobacter pylori* a cause of gastric cancer? An appraisal of the seroepidemiological evidence. *Cancer Epidemiol Biomarkers Prev* **3:** 445 51.

Palli D, Decarli A, Cipriani F *et al* (1991). Plasma pepsinogens, nutrients, and diet in areas of Italy at varying gastric cancer risk. *Cancer Epidemiol Biomarkers Prev* **1:** 45 50.

Palli D, Decarli A, Cipriani F *et al* (1993). *Helicobacter pylori* antibodies in areas of Italy at varying gastric cancer risk. *Cancer Epidemiol Biomarkers Prev* **2:** 37 40.

Parsonnet J, Friedman GD, Vandersteen DP, Vogelman JH, Orentreich N, Sibley RK (1991). *Helicobacter pylori* infection and the risk of gastric carcinoma. *N Engl J Med* **325:** 1127 31.

Pobel D, Riboli E, Cornée J, Hémon B, Guyader M (1995). Nitrosamine, nitrate and nitrite in relation to gastric cancer: a case control study in Marseille, France. *Eur J Epidemiol* **11:** 67 73.

Preussmann R (1984). Occurrence and exposure to *N*-nitroso compounds and precursors. In *N-nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer* (IARC Sci Publ No. 57; eds O'Neill IK, Von Borstel RC, Miller CT, Long J, Bartsch H); IARC, Lyon, pp. 3 14.

Reed PI, Smith PLR, Haines H, House FR, Walters CL (1981). Effect of cimetidine on gastric juice *N*-nitrosamine concentration. *Lancet* **2:** 553 6.

Risch HA, Jain M, Choi NW *et al* (1985). Dietary factors and the incidence of cancer of the stomach. *Am J Epidemiol* **122:** 947 59.

Sierra R, Chinnock A, Ohshima H *et al* (1993). *In vivo* nitrosoproline formation and other risk factors in Costa Rican children from high- and low-risk areas for gastric cancer. *Cancer Epidemiol Biomarkers Prev* **2:** 563 8.

World Health Organisation (1978). *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Some N-nitroso Compounds*. Volume 17, IARC, Lyon.

Xu GP, Reed PI (1993). *N*-nitroso compounds in fresh gastric juice and their relation to intragastric pH and nitrite employing an improved analytical method. *Carcinogenesis* **14:** 2547 51.

Zatonski W, Ohshima H, Przewozniak K *et al* (1989). Urinary excretion of *N*-nitrosamino acids and nitrate by inhabitants of high- and low-risk areas for stomach cancer in Poland. *Int J Cancer* **44:** 823 7.